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☐ *Additional inventors are being named on the separately numbered sheets attached hereto*

TITLE OF THE INVENTION (280 characters max)

CALORIMETER AND METHODS OF USE THEREOF

Direct all correspondence to: **CORRESPONDENCE ADDRESS**

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CALORIMETER AND METHODS OF USE THEREOF

FIELD OF THE INVENTION

[0001] The present invention is in the field of devices for measuring heat absorbed or generated from various chemical, biochemical, physical, light-induced, and biological processes.

BACKGROUND OF THE INVENTION

[0002] Microcalorimeters are devices that measure very small quantities of heat. In chemistry, biochemistry, cell biology, and pharmacology, ultrasensitive microcalorimeters are frequently used to measure thermodynamic properties of biological macromolecules, such as proteins.

[0003] Two commonly used types of microcalorimeters are the differential scanning calorimeter and the isothermal titration calorimeter. The differential scanning calorimeter automatically raises or lowers the temperature of the system at a given rate, while monitoring any temperature differential that arises between the two cells. From the temperature differential information, small differences between the amount of heat absorbed or released by the sample cell in comparison to the reference cell can be determined and attributed to the test substance.

[0004] In isothermal titration calorimetry, the instrument maintains a constant temperature while the concentration of an additional substance added to the cells is varied. The additional substance can be, e.g., a ligand that binds to the test substance in the sample cell. The instrument measures the heat absorbed or released as the newly introduced ligand binds to the test substance. By repeating the titration experiment using multiple additions of the ligand until binding is complete, various information concerning the interaction between the test substance and the ligand, e.g., stoichiometry, binding constant, and heat of binding, can be determined.

[0005] There is a need in the art for improved calorimetry devices and methods. The present invention addresses this need.

Literature

[0006] U.S. Patent No. 6,513,969; U.S. Patent No. 6,193,413.

SUMMARY OF THE INVENTION

[0007] The present invention provides a calorimeter device, generally comprising a reaction vessel which may be U-shaped or cantilevered; and a sensor for detecting temperature changes. In various embodiments, the sensor detects heat input into or output from the reaction vessel; changes in the electrical properties of a material coated onto the reaction vessel; changes in the mechanical properties of the reaction vessel; or changes in the resonance properties of the reaction vessel. The present invention further provides arrays of a subject calorimeter device. The present invention further provides methods of detecting a temperature change that occurs as a result of a chemical, biochemical, biological, light-induced, or physical process. The methods generally involve introducing a sample into a subject device, and detecting a temperature change.

BRIEF DESCRIPTION OF THE DRAWINGS

[0008] Figures 1-5 depict exemplary embodiments of the invention.

[0009] Before the present invention is further described, it is to be understood that this invention is not limited to particular embodiments described, as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting, since the scope of the present invention will be limited only by the appended claims.

[0010] Where a range of values is provided, it is understood that each intervening value, to the tenth of the unit of the lower limit unless the context clearly dictates otherwise, between the upper and lower limit of that range and any other stated or intervening value in that stated range, is encompassed within the invention. The upper and lower limits of these smaller ranges may independently be included in the smaller ranges, and are also encompassed within the invention, subject to any specifically excluded limit in the stated range. Where the stated range includes one or both of the limits, ranges excluding either or both of those included limits are also included in the invention.

[0011] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those

described herein can also be used in the practice or testing of the present invention, the preferred methods and materials are now described. All publications mentioned herein are incorporated herein by reference to disclose and describe the methods and/or materials in connection with which the publications are cited.

[0012] It must be noted that as used herein and in the appended claims, the singular forms "a", "and", and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a reaction" includes a plurality of such reactions and reference to "the device" includes reference to one or more devices and equivalents thereof known to those skilled in the art, and so forth.

[0013] The publications discussed herein are provided solely for their disclosure prior to the filing date of the present application. Nothing herein is to be construed as an admission that the present invention is not entitled to antedate such publication by virtue of prior invention. Further, the dates of publication provided may be different from the actual publication dates which may need to be independently confirmed.

DETAILED DESCRIPTION OF THE INVENTION

[0014] The present invention provides a calorimeter device, generally comprising a reaction vessel (also referred to herein as "calorimeter vessel," "calorimeter tube," "fluidic channel," or "tube"), which reaction vessel may be U-shaped; and a sensor for detecting temperature changes. In various embodiments, the sensor detects heat input into or output from the reaction vessel; changes in the electrical properties of a material coated onto the reaction vessel; changes in the mechanical properties of the reaction vessel; or changes in the resonance properties of the reaction vessel. The present invention further provides arrays of a subject calorimeter device. The present invention further provides methods of detecting a temperature change that occurs as a result of a chemical, biochemical, biological, light-induced, or physical process. The methods generally involve introducing a sample into a subject device, and detecting a temperature change in the device, which temperature change is representative of a calorimetric effect of the process involving the sample.

CALORIMETER DEVICE

[0015] The present invention provides a calorimeter device, generally comprising a reaction vessel (which may be U-shaped or cantilevered); and a sensor for detecting a temperature change in the reaction vessel. A subject calorimeter device is useful for detecting temperature changes that occur during the course of various processes, including a chemical reaction, a biochemical reaction, a biological event, a light-induced process, and a physical process.

[0016] The temperature change is in some embodiments detected by a change in mechanical property of the reaction vessel, e.g., by detecting bending of the reaction vessel. The temperature change is in other embodiments detected by changes in electrical properties of a layer coating the reaction vessel. The temperature change is in other embodiments detected by a change in resonance properties of the reaction vessel. The temperature change is in other embodiments detected by the heating or cooling necessary to maintain the tube at a constant temperature; the heat applied or decreased is a direct measure of the heat evolved or absorbed by the chemical, light-induced, biological, biochemical, or physical process occurring in the liquid.

[0017] The reaction vessel is a thin-walled, low-volume enclosure through which one or more liquids can be injected. The reaction vessel includes an inlet and an outlet. A sample is introduced into the inlet. Typically, the reaction vessel is in the form of a tube or other fluidic channel. The shape of the tube or other channel generally provides for laminar flow of a liquid through the tube. Typically, the reaction vessel is mounted on a support, where the support points are generally at the inlet and the outlet of the reaction vessel. The reaction vessel is shaped such that the above-noted changes (e.g., mechanical bending, changes in electrical properties, changes in resonance frequency, etc.) can be detected. As such, suitable shapes include, but are not limited to, a U-shaped configuration.

[0018] The reaction vessel has at least a minimum length such that the inlet and outlet can be mounted onto the same surface of a support (e.g., a support block). As such, the reaction vessel generally has a length of from about 0.5 cm to about 2 cm.

[0019] The reaction vessel has an inner diameter of from about 10 μm to about 1 mm, e.g., from about 10 μm to about 50 μm, from about 50 μm to about 100 μm, from about 100 μm to about 500 μm, or from about 500 μm to about 1 mm.

[0020] The total volume capacity of the reaction vessel is in a range of from about 1 μl to about 1 ml, e.g., from about 1 μl to about 10 μl, from about 10 μl to about 100 μl, from about 100 μl to about 500 μl, or from about 500 μl to about 1 ml.

[0021] The reaction vessel wall is thin, e.g., in a range of from about 1 μm to about 1 mm, e.g., from about 1 μm to about 10 μm, from about 10 μm to about 100 μm, from about 100 μm to about 500 μm, or from about 500 μm to about 1 mm.

[0022] A subject device detects temperature changes in the picoJoule (pJ) range. For example, a subject device detects temperature changes in the range of from about 1 pJ to about 1000 pJ, e.g., from about 1 pJ to about 10 pJ, from about 10 pJ to about 50 pJ, from about 50 pJ to about 100 pJ, from about 100 pJ to about 200 pJ, from about 200 pJ to about 250 pJ, from about 250 pJ to about 500 pJ, from 500 pJ to about 750 pJ, or from about 750 pJ to about 1000 pJ.

[0023] The reaction vessel comprises materials that do not react with, or interfere with, any process taking place in the reaction vessel, e.g., the materials are generally inert. In many embodiments, the reaction vessel comprises a material such a silicon, a silicon nitride, and the like. In some embodiments, the reaction vessel comprises one or more layers of materials.

[0024] In some embodiments, the reaction vessel comprises a layer that functions as a sensor ("a sensor layer") to determine the temperature of the reaction vessel, or to act as an actuated thermo-mechanical transducer. In other embodiments, the reaction vessel comprises a layer that functions as a sensor, such that a temperature change is detected as a change in the electrical properties of the layer. In some embodiments, the layer is a bimetallic layer or other material that bends in response to a change in temperature. In some embodiments, the material is a shape-memory material (e.g., a nickel-titanium alloy; NITINOL).

[0025] In some embodiments, the sensor layer is a thermistor. Suitable thermistors include those with a negative resistance/temperature coefficient (NTC) and those with a positive resistance/temperature coefficient (PTC). NTC thermistors include those manufactured from the oxides of the transition metals, e.g., manganese, cobalt, copper and nickel. PTC thermistors include those manufactured from barium titanate and strontium titanate. Suitable NTC and PTC thermistors and thermistor materials include those discussed in U.S. Patent Nos. 6,607,679, 6,218,928, and 6,712,771.

[0026] In some embodiments, the sensor layer is a piezoelectric material, or a piezoresistive material. These materials will produce an electric field when the material changes dimensions, e.g., when the reaction vessel bends. Piezoelectric materials are known in the art; see, e.g., "Piezoelectric Materials: Advances in Science, Technology and Applications (2000) C. Galassi et al., eds., Kluwer Academic Publishers. Suitable piezoelectric materials include quartz (SiO_2), barium titanate ($BaTiO_2$), ST-cut quartz, quartz crystals, piezoelectric ceramics, such as those of the barium titanate and lead zirconium titanate families, e.g., $LiNbO_3$; $BaTiO_3$; 95 wt. % $BaTiO_3$/5% $GaTiO_3$; 80 wt. % $BaTiO_3$ /12% $PbTiO_3$/8% $CaTiO_3$; $PbNb_2O_6$; $Na_{0.5}K_{0.5}NbO_3$; and the like. In some cases, the sensor layer may comprise a piezoelectric coating material, such as ZnO or AlN, applied to a non-piezoelectric material, such as silicon. The piezoelectric properties of these and other suitable materials are provided in CRC Handbook of Materials Science, Vol. III, Charles T. Lynch, CRC Press: Boca Raton, 198 (1975).

Detecting resonance

[0027] In some embodiments, the reaction vessel is embedded in a micromechanical cantilever. In some of these embodiments, the cantilever comprises two layers of materials such as silicon and aluminum.

[0028] In some embodiments, e.g., wherein the U-shaped reaction vessel is mounted in a vacuum exterior, the Q factor of the cantilever in resonance is used as a very sensitive thermal sensor. The high Q factor of the cantilever provides a means to determine the resonance frequency as well as the internal damping energy of the liquid in the tube. In this embodiment, no bimetallic layer is required. The temperature changes occurring as a result of a process taking place within the reaction vessel has two effects: first, the elongation of the tube due to change in the mass of the thermally expanded liquid will change the resonance frequency. Likewise, the viscosity of water changes by approximately 0.2% per degree Celsius. This will change the Q factor. Other processes occurring within the tube, such as nucleic acid hybridization, can also influence the Q-factor and resonance properties of the resonating tube.

Detecting temperature changes

[0029] In some embodiments, the sensor detects a change in temperature in the reaction vessel. In some of these embodiments, isothermal conditions are maintained by use of

an integrated heating device. The integrated heating device in some embodiments also functions as a temperature sensing element and/or a thermo-mechanical transducer.

[0030] In some embodiments, the reaction vessel is heated by application of an electrical current through a coating layer. The reaction vessel is heated or cooled to maintain a constant temperature. The amount of heat applied or decreased is a direct measure of the heat evolved or absorbed during the process taking place in the reaction vessel. Thus, e.g., where the process taking place in the reaction vessel generates heat, the amount of heat generated during the process is determined by the degree of cooling necessary to maintain the reaction vessel at a constant temperature. Conversely, where the process taking place in the reaction vessel absorbs heat, the amount of heat absorbed during the process is determined by the amount of applied heat required to maintain the reaction vessel at a constant temperature.

[0031] In some embodiments, the device is enclosed in a vacuum environment, to minimize convection and/or conduction heat losses that would occur in atmospheric conditions.

Detecting changes in mechanical properties of the reaction vessel

[0032] In some embodiments, a temperature change in the reaction vessel is detected by changes in mechanical properties of the reaction vessel. For example, in some embodiments, the reaction vessel bends in response to a temperature change in the reaction vessel. In these embodiments, the reaction vessel comprises one or more materials that bend in response to a temperature change. Such materials are well known to those skilled in the art and include, but are not limited to, piezoresistive materials, bimetallic materials, and the like. Such a micromechanical sensor can be provided where the reaction vessel is coated with metal on one side, which metal undergoes bending due to differential thermal expansion of the coating metal and the material onto which the sensor layer is coated (the "bimetallic effect"). Thus, in many embodiments, the coating layer comprises a thermal sensitive material.

[0033] Sensors to detect a mechanical response to a temperature change include, but are not limited to, capacitative sensing, piezoelectric effect, beam deflection, interferometric sensing methods, optical beam reflection, and electron tunneling sensors.

[0034] Non-limiting examples of these embodiments are depicted in Figures 3 and 4. For example, in some embodiments, a subject device comprises a reflector mounted on the reaction vessel, where the reflector (e.g., a mirror) reflects a beam of incident light. Movement of the mirror in response to a temperature change in the reaction vessel is detected by a sensor which detects the reflected beam of light.

[0035] In these embodiments, light, e.g., a laser beam, is directed onto a mirror mounted on the reaction vessel, and the position of a reflected laser beam is detected by a sensor. For example, a first position of a laser beam reflected by a mirror mounted on the reaction vessel is detected at a first time; and a second position of a laser beam reflected by the mirror is detected at a second time. Where the second position differs substantially from the first position indicates bending of the reaction vessel, and hence indicates a change in temperature in the reaction vessel.

[0036] Measurements temperature changes in the vessel are made at regular intervals (e.g., every 5 seconds, 10 seconds, 20 seconds, 30 seconds, 60 seconds, two minutes, 5 minutes, 10 minutes, 15 minutes, etc.); or substantially continuously. Alternatively, measurements of temperature changes in the vessel are made at a single time point, or at random time points.

[0037] Suitable sensors for detecting a reflected beam of light include any device that is capable of detecting a reflected beam of light. Suitable sensors include, but are not limited to, charge coupled devices (CCD). The CCD camera is connected to an image analysis computer system for data storage and analysis.

[0038] In other embodiments, bending of the reaction vessel (indicating a temperature change within the reaction vessel) is detected by use of a capacitor (see, e.g., Figure 4). For example, a thin, flat surface mounted on the reaction vessel functions as one side of an electrical capacitor for distance determination (e.g., measuring bending of the reaction vessel). In operation, the bending of the tube is kept constant by adjusting the applied heating power, which is absorbed by the calorimeter tube.

Detecting changes in electrical properties of the coating layer of the reaction vessel

[0039] In some embodiments, a temperature change is detected by detecting an electrical change in a material layered onto the reaction vessel. Suitable materials include piezoelectric materials and piezoresistive materials, as discussed above.

Mixing

[0040] Mixing of reactants in the reaction vessel can be achieved by any of a variety of means. As one non-limiting example, reactants A and B are injected into the inlet of a reaction vessel; reactants A and B do not mix due to turbulence, but instead exhibit laminar flow. In this example, the reaction is diffusion controlled. The tube can be patterned to control mixing. Alternatively, turbulence can be introduced into the tube to effect mixing.

[0041] Mixing can also be achieved by increasing the flow speed exceeding the Reynolds number for turbulent flow. Optimal flow conditions can be determined by observing the mixing of liquid dyes. Injected reactants can also be pulsed in the tube.

Exemplary embodiments

[0042] Figures 1-5 depict exemplary embodiments of a subject device. Figure 1 depicts a reaction vessel in the form of a tube **10** or other fluidic channel, supported at one or more points. Tube **10** has inlet **11** into which a liquid comprising one or more reactants, cells, biomolecules, etc. can be injected; and outlet **12**. The liquid in the tube may change temperature due to a variety of processes such as a chemical reaction, a biochemical reaction, a biological reaction, or a light-induced process. A sensor detects a temperature change. In various embodiments, the sensor detects heat input or output; changes in the electrical properties of a material coated onto the inner surface of the reaction vessel; or changes in the mechanical properties of the reaction vessel.

[0043] Figure 2 depicts an exemplary embodiment of the invention, in which the U-shaped reaction vessel **10** is embedded in, or formed within, a micromechanical cantilever structure **20** made from two layers of material, such as silicon (Si) and aluminum (Al).

[0044] Figure 3 depicts an exemplary embodiment of the invention, in which the U-shaped reaction vessel **10** is mounted on a block **30** that thermalizes the liquid entering the reaction vessel. Two inlet lines **31** are mounted on top of the block; and an outlet line **32** and flush line **33** are mounted below. The block contains a micro valve system that opens and closes the inlet lines, where the valve system is coupled to a flowmeter which is controlled by an electrical signal line **34**. A small mirror **40** on the end of the tube **10** is used in conjunction with standard beam deflection techniques to measure the beam deflection. Contact pads **35** for thermistor or bimetallic heating are positioned

next to the reaction vessel. A laser beam **50** is shown, with incident beam **51** transmitted from a light source, and reflected beam **52** reflected by mirror **40**. The device **60** is in some embodiments provided in an array **70**, which enables references, standards, and other differential modes of operation in parallel.

[0045] Figure 4 depicts an exemplary embodiment of the invention, in which two electrodes **81** form a capacitor **80** which is used to detect bending of the tube **10**. The electrical heater element **82** is adjusted to maintain a constant gap.

[0046] Figure 5 depicts an exemplary embodiment of the invention, in which a device **60** includes two reservoirs **90** of liquid, with valves for automated analysis.

ARRAYS

[0047] The present invention further provides arrays of a subject calorimeter device. An array comprises two, three, four, five, six, seven, eight, nine, ten, from 10 to 25, from 25 to 50, or from 50 to 100, or more, subject devices. In many embodiments, a subject array includes a reference reaction vessel; and one or more standards, e.g., where one or more subject devices provide for generating a standard curve against which to evaluate the results from a test sample. An array is provided in any of a variety of configurations.

[0048] A subject array is useful for screening a variety of compounds, e.g., in the setting of proteomic analyses; small molecule drug discovery; and the like.

[0049] In some embodiments, a subject array further comprises a data storage and analysis means and a computer readable medium for storing data generated by a subject array.

UTILITY

[0050] A subject device is useful for detecting temperature changes that occur during the course of various processes, including a chemical reaction, a biochemical reaction, a biological event, a light-induced process, and a physical process. The temperature change that occurs is a readout for the chemical reaction, biochemical reaction, biological event or status, light-induced process, or physical process.

[0051] In some embodiments, a subject calorimeter device is used as a photothermal spectrophotometer or IR-spectrophotometer, based on the thermal signal generated by infrared (IR), visible, or ultraviolet (UV) absorption.

[0052] A subject device enables the variations in thermal properties such as a phase transition, by ramping the temperature Differential Scanning Calorimetry. The pressure applied in the tube can be used in combination with the above methods to examine folding properties of large biopolymers.

[0053] A subject device is useful for detecting chemical reactions. A subject device is useful for detecting biochemical reactions. A subject device is useful for detecting binding of a small molecule to a macromolecule (e.g., a polypeptide, a polynucleotide, a polysaccharide, a lipid). For example, binding of a small molecule (e.g., a molecule having a molecular weight in the range of from about 50 daltons to about 5,000 daltons, or from about 50 daltons to about 2,5000 daltons) to a polypeptide (e.g., a receptor, an enzyme, and the like) is useful for identifying agents (e.g., pharmaceutically active agents) that modulate (e.g., increase or decrease) the activity of a polypeptide. A subject device is useful for detecting binding between two macromolecules. For example, a subject device is useful for detecting nucleic acid hybridization; for detecting binding of a protein to a nucleic acid; for detecting binding of two proteins to one another; etc.

Determining a characteristic of a macromolecule

[0054] In some embodiments, the invention provides a method of determining a characteristic of a macromolecule, the method involving introducing the macromolecule into the reaction vessel of a subject device; and detecting a temperature change in the reaction vessel. The characteristic is in some embodiments protein conformation, where the protein conformation in various solvents, or in the presence of various analytes or other macromolecules, is determined. The characteristic is in some embodiments binding to a small molecule or binding to a macromolecule or a cell. The characteristic is in some embodiments a biological function, e.g., enzymatic activity, in the presence of various solvents, analytes, agonists, antagonists, or macromolecules.

Detecting cancerous cells

[0055] When cells become cancerous and metastasize, the mechanical properties of their membranes changes. The present invention provides a method of detecting mechanical properties of mammalian cells. Cells flowing through a reaction vessel in a subject device in a vacuum; the frequency-dependent resonance and Q factor are recorded; and the Q factor provides an indication of the local damping of the cell.

[0056] The cells are pumped in laminar flow through the tube one by one. An optical detector senses a cell entering the inlet of the tube when the cell is at the apex of the U-shaped tube and when it exits. Three optical sensors are used. The tube is in a vacuum environment and is mechanically vibrated over a range of frequencies ($H_2 \rightarrow 100Kh_2$). The mechanical response is measured from the motion of the tube capability piezo electrically or optically.

[0057] The power spectrum then provides the Q factor of the medium in the tube, scans are made when the cell is detected at the apex, the other two sensors coupled with flow control ensure only one cell is measured at a time. Cancerous cells are diverted by standard cell sorting techniques from healthy ones. The device can also be used to study the action of drugs that "harden" (e.g., return the cell to a non-cancerous state) cancerous cells but not adversely affect or otherwise influence healthy ones.

[0058] Many other uses are possible, e,g., therapy where, e.g., cells taken from an individual are treated, then returned to the individual.

[0059] The invention further provides a method of treating a disease or disorder. The methods generally involve identifying a cancerous cell using a subject method; and recommending a treatment regimen appropriate to the abnormality.

[0060] For example, where a cell in a tissue biopsy is determined to be a cancerous cell, a treatment regimen appropriate to the particular type of cancer is recommended. In some embodiments, the methods provide for staging of the cancer. A course of chemotherapy or radiation therapy appropriate to the stage of the cancer is then recommended.

Detecting the presence of an analyte

[0061] The invention further provides assays for detecting the presence of an analyte in a test sample. The methods generally involve contacting a molecular entity (e.g., a polypeptide, a polynucleotide, a carbohydrate, a polysaccharide) with a test sample; and detecting any change in the temperature of the reaction vessel in response to an interaction between the molecular entity and the test sample. Such a screening assay is useful to detect the presence in a sample of an analyte suspected to exist in the sample, e.g., a subject screening assay can be used to detect the presence in a sample of a toxin or a toxic bacterium, e.g., an environmental agent (e.g., a pesticide, an herbicide, an

environmental toxin, and the like), an agent of chemical or biological warfare (e.g., nerve gas, anthrax, etc.).

[0062] Assays of the invention include controls, where suitable controls include a sample (e.g., an analyte) in the absence of the test sample. Generally a plurality of assay mixtures is run in parallel with different known concentrations of the analyte being detected to obtain a differential response to the various concentrations. Typically, one of these concentrations serves as a negative control, i.e. at zero concentration or below the level of detection. The assay methods provide for qualitative (e.g., presence or absence), semi-quantitative, and quantitative detection of analyte.

Screening assays

[0063] The invention provides screening assays for identifying agents that have pharmaceutical activity. The methods generally involve introducing a test agent and a second compound into the reaction vessel of a subject device; and determining the change if any, in the temperature of the liquid in the reaction vessel. The invention further provides assays for detecting the presence of an analyte in a test sample. The methods generally involve contacting a molecular entity (e.g., a polypeptide, a polynucleotide, a ligand, etc.) or a cell with a test sample; and detecting any change in temperature in the reaction vessel in response to the test sample.

[0064] The terms "candidate agent," "agent", "substance" and "compound" are used interchangeably herein. Test agents encompass numerous chemical classes, typically synthetic, semi-synthetic, or naturally-occurring inorganic or organic molecules. Test agents may be small organic compounds having a molecular weight of more than 50 and less than about 2,500 daltons, or less than about 5,000 daltons. Test agents may comprise functional groups necessary for structural interaction with proteins, particularly hydrogen bonding, and may include at least an amine, carbonyl, hydroxyl or carboxyl group, and may contain at least two of the functional chemical groups. The test agents may comprise cyclical carbon or heterocyclic structures and/or aromatic or polyaromatic structures substituted with one or more of the above functional groups. Test agents are also found among biomolecules including peptides, saccharides, fatty acids, steroids, purines, pyrimidines, derivatives, structural analogs or combinations thereof.

[0065] Test agents include those found in large libraries of synthetic or natural compounds. For example, synthetic compound libraries are commercially available from Maybridge Chemical Co. (Trevillet, Cornwall, UK), ComGenex (South San Francisco, CA), and MicroSource (New Milford, CT). A rare chemical library is available from Aldrich (Milwaukee, Wis.). Alternatively, libraries of natural compounds in the form of bacterial, fungal, plant and animal extracts are available from Pan Labs (Bothell, WA) or are readily producible. Additionally, natural or synthetically produced libraries and compounds are readily modified through conventional chemical, physical and biochemical means, and may be used to produce combinatorial libraries. Libraries of test agents also include cDNA libraries, e.g., expression libraries from a given cell type, from a cell in response to an agent, from a cell of a given physiological status (e.g., a cancerous cell), and the like.

[0066] Known pharmacological agents may be subjected to directed or random chemical modifications, such as acylation, alkylation, esterification, amidification, etc. to produce structural analogs. New potential therapeutic agents may also be created using methods such as rational drug design or computer modeling.

[0067] Assays of the invention include controls, where suitable controls include a sample (e.g., a cell sample) in the absence of the test agent. Generally a plurality of assay mixtures is run in parallel with different agent concentrations to obtain a differential response to the various concentrations. Typically, one of these concentrations serves as a negative control, i.e. at zero concentration or below the level of detection.

[0068] Agents that have an effect in an assay method of the invention may be further tested for cytotoxicity, bioavailability, and the like, using well known assays. Agents that have an effect in an assay method of the invention may be subjected to directed or random and/or directed chemical modifications, such as acylation, alkylation, esterification, amidification, etc. to produce structural analogs. Such structural analogs include those that increase bioavailability, and/or reduced cytotoxicity. Those skilled in the art can readily envision and generate a wide variety of structural analogs, and test them for desired properties such as increased bioavailability and/or reduced cytotoxicity and/or ability to cross the blood-brain barrier.

[0069] The components of the assay mixture are added (e.g., injected into the reaction vessel) in any order that provides for the requisite binding or other activity. Incubations are performed at any suitable temperature, typically between 4°C and 40°C. Incubation periods are selected for optimum activity, but may also be optimized to facilitate rapid high-throughput screening. Typically between 0.1 and 1 hour will be sufficient.

[0070] While the present invention has been described with reference to the specific embodiments thereof, it should be understood by those skilled in the art that various changes may be made and equivalents may be substituted without departing from the true spirit and scope of the invention. In addition, many modifications may be made to adapt a particular situation, material, composition of matter, process, process step or steps, to the objective, spirit and scope of the present invention. All such modifications are intended to be within the scope of the claims appended hereto.

CLAIMS

What is claimed is:

1. A calorimetric device comprising

a) a U-shaped reaction vessel having an inlet and an outlet, and mounted onto a support at or near the inlet and the outlet; and

b) a sensor.

2. The device of claim 1, wherein the sensor detects temperature input into the reaction vessel and/or temperature output from the vessel required to maintain the reaction vessel at a substantially constant temperature.

3. The device of claim 1, further comprising a coating layer on the reaction vessel, wherein the coating layer provides for mechanical bending of the reaction vessel in response to a temperature change within the reaction vessel.

4. The device of claim 1, further comprising a coating layer on the reaction vessel, wherein the coating layer provides a means of detecting a change in electrical properties of the coating layer in response to a temperature change within the reaction vessel.

5. The device of claim 1, further comprising a mirror mounted onto the reaction vessel.

6. A method of detecting a chemical reaction, a biochemical reaction, a physical process, a light-induced process, or a biological reaction, the method comprising

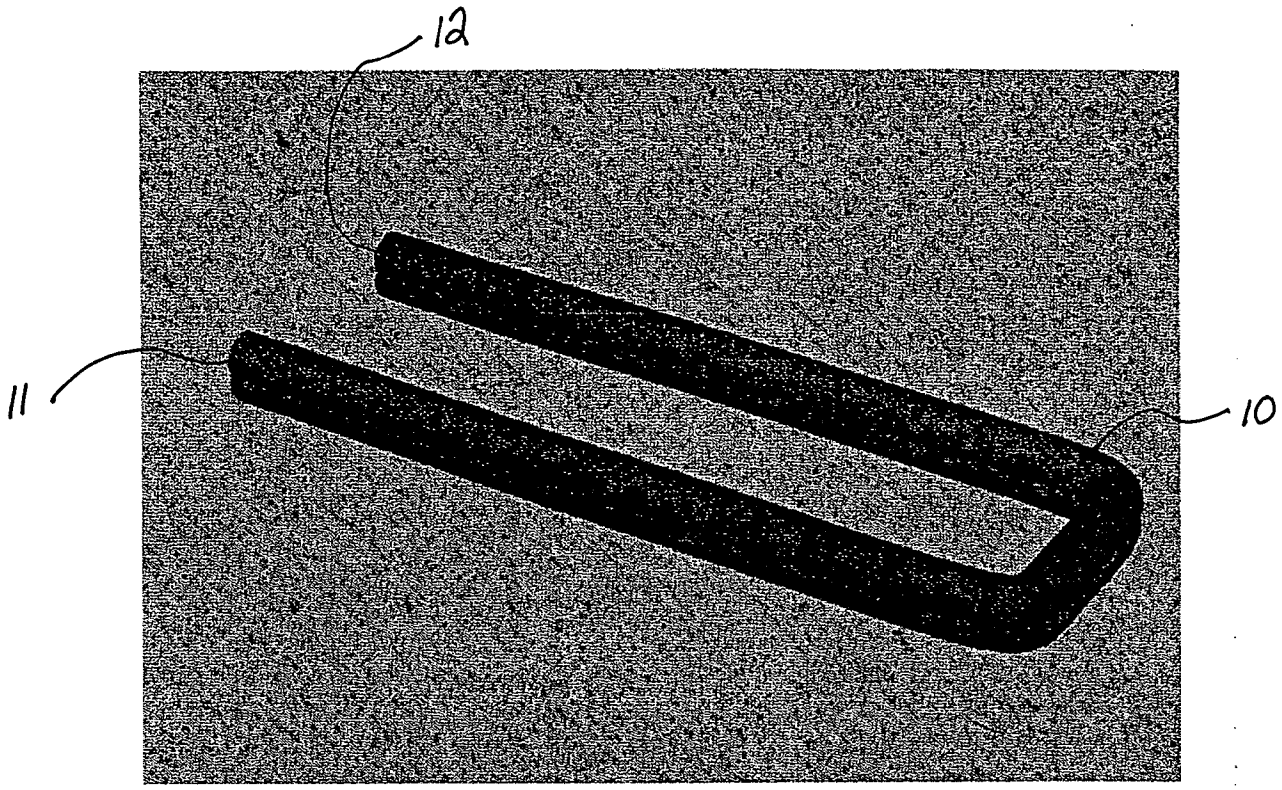
introducing a sample comprising a chemical reactant, a biological entity, or a macromolecule into a subject device; and

detecting a temperature change in the reaction vessel.

CALORIMETER AND METHODS OF USE THEREOF

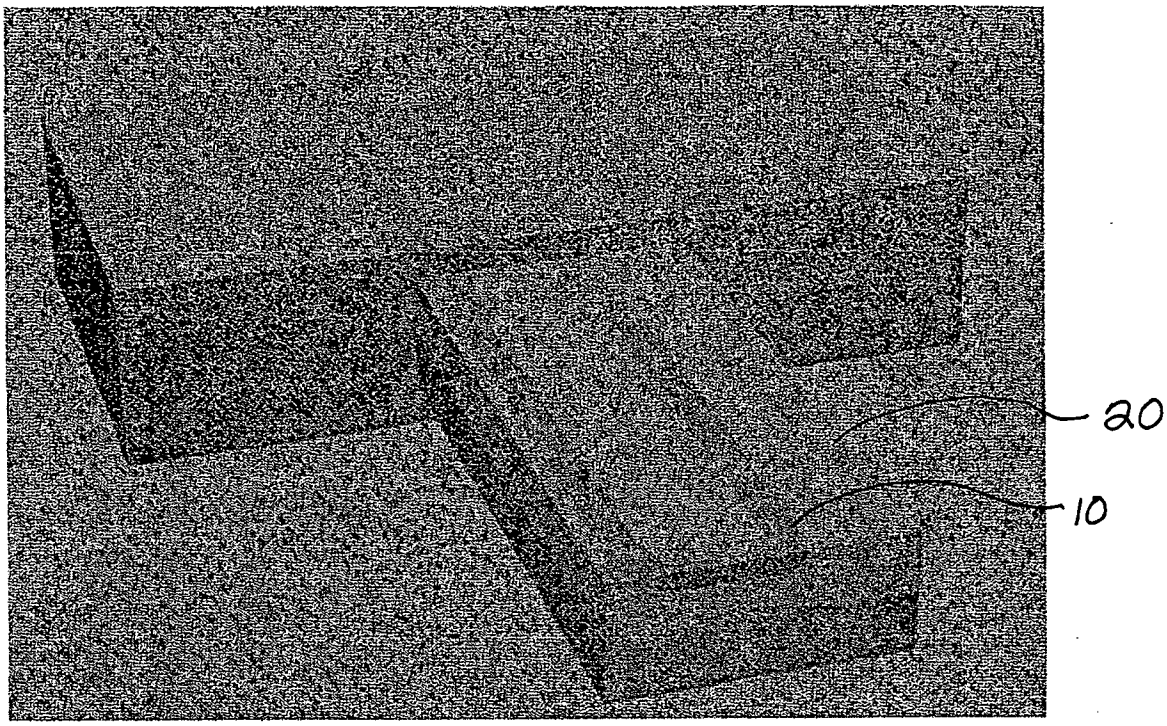
ABSTRACT OF THE DISCLOSURE

The present invention provides a calorimeter device, generally comprising a reaction vessel which may be U-shaped or cantilevered; and a sensor for detecting temperature changes. In various embodiments, the sensor detects heat input into or output from the reaction vessel; changes in the electrical properties of a material coated onto the reaction vessel; changes in the mechanical properties of the reaction vessel; or changes in the resonance properties of the reaction vessel. The present invention further provides arrays of a subject calorimeter device. The present invention further provides methods of detecting a temperature change that occurs as a result of a chemical, biochemical, biological, light-induced, or physical process. The methods generally involve introducing a sample into a subject device, and detecting a temperature change.



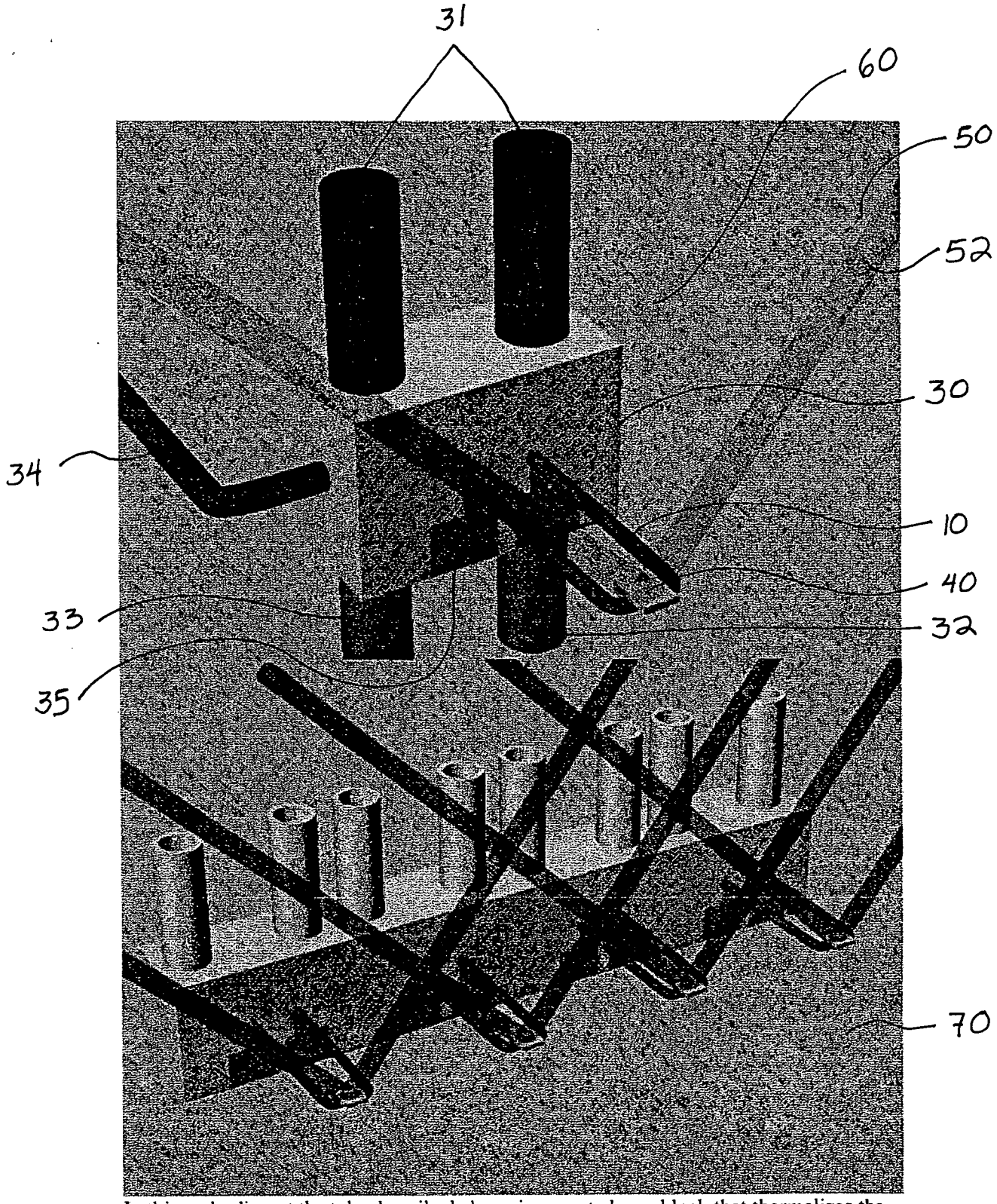
Principle of the calorimeter: A tube or other fluidic channel is support at one or more points. The liquid in the tube may change temperature due to a variety of processes such as chemical reaction or light induced process. The temperature change is determined from a coating layer which introduces bending vai the bimetallic process (red layer) or the layer may act as a thermistor. The tube may also heated by application of an lectrical current through the layer. In the preferred embodiment the tube is heated (or cooled slightly)to maintain a constant temperature. The current is changed to maintain a constant deflection or thermistor resistance. The heat applied or decreased in this way is a direct measure of the heat evolved or absorbed by the chemical of physical process occurring in the liquid.

Figure 1



Here the tube is embedded in a micromechanical cantilever made from two layers of material such as Si (green) and Aluminum (yellow). The channel is fabricated in the middle. This embodiment operates on similar principles to the tube embodiment.

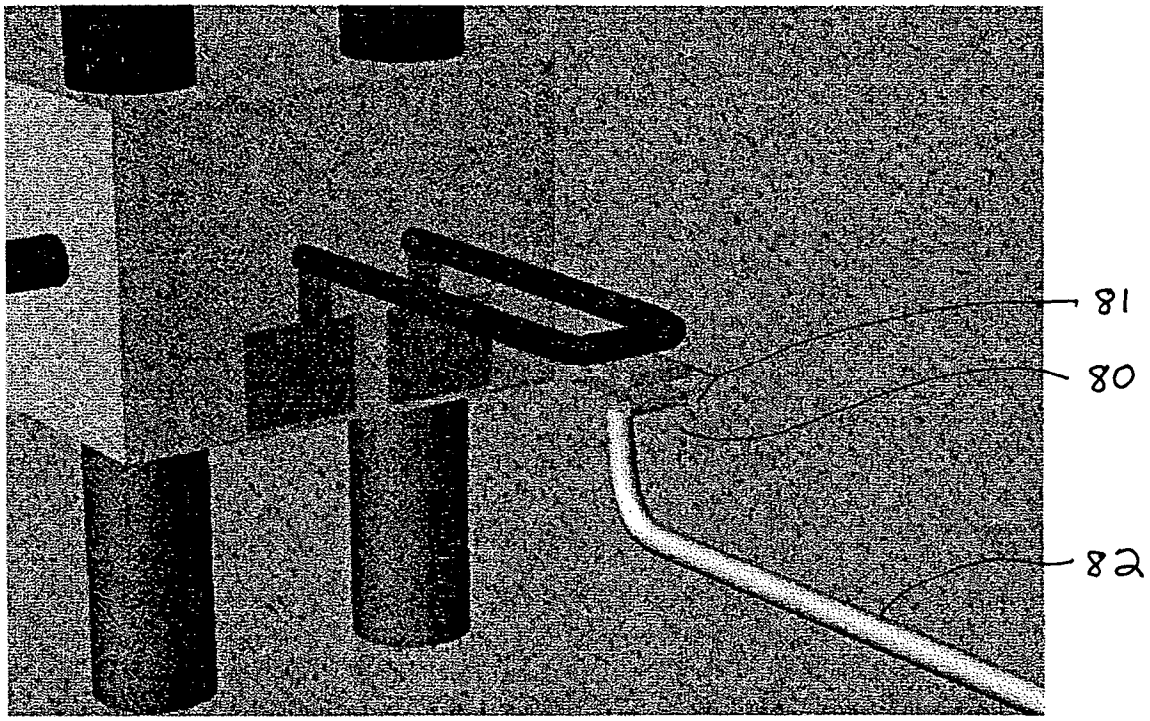
Figure 2



In this embodiment the tube described above is mounted an a block that thermalizes the liquid entering the tube. Two inlet lines are mounted on top of the block (blue) and an outlet line and flush line are mounted below (green). The block contained a micro valve

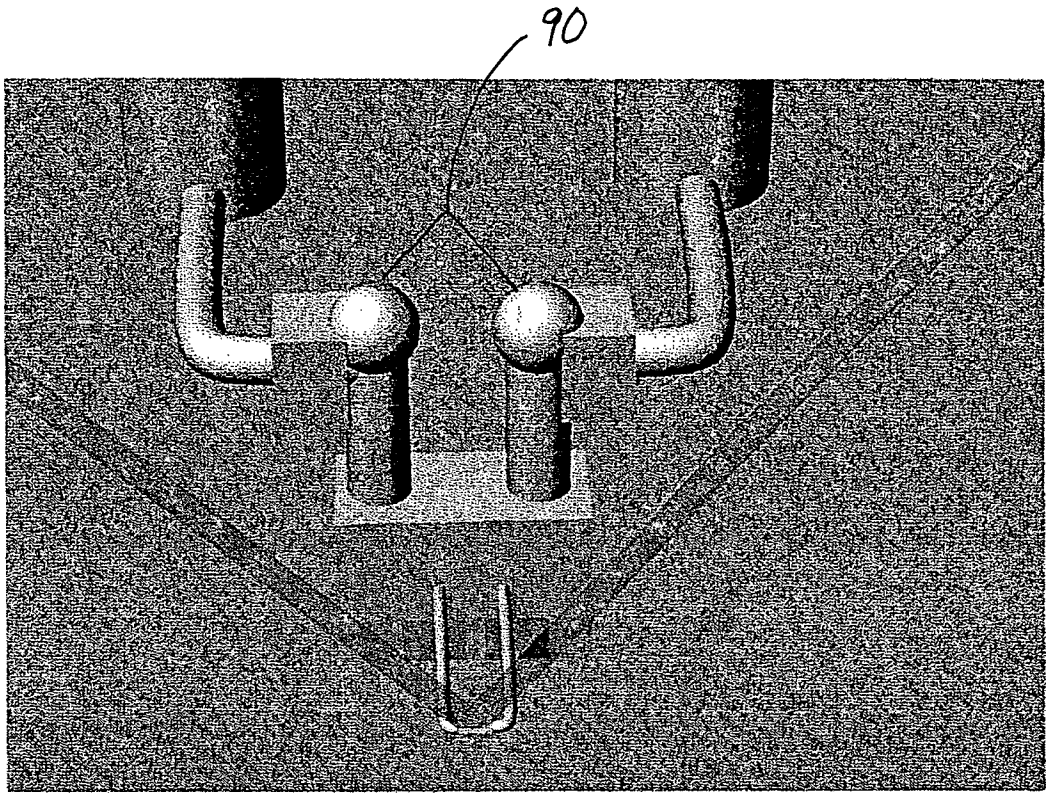
Figure 3

system opening or closing the fourin;let lines coupled to a flowmeter which is controlled by an electrical signal line shown in red. A small mirror on the end of the tube is used in conjunction with standard beam deflection techniques to measure the beam deflection. Contact pad for thermistor or bimetallic heating are in orange. The laser beam is shown. The device may be used in an array form which enables references, standards and other differential modes of operation in parallel as shown below the single embodiment



In this embodiment the two yellow electrodes form a capacitor which is used to determine the bending of the tube. The electrical heater element is adjusted to maintain constant gap

Figure 4



System with two reservoirs of liquid, right and left and valves for automated analysis

Figure 5

Figure 5

APPLICATION INFORMATION

Application Type::	Provisional
Title::	CALORIMETER AND METHODS OF USE THEREOF
Attorney Docket Number::	UCLA-013PRV
Request for Non-Publication?::	
Assignee for Publication::	
Total Drawing Sheets::	Five
Small Entity?::	Small
License US Govt. Agency::	No
Contract or Grant Numbers::	
Sequence Submission?::	No
Computer Readable Form (CRF)?::	

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CONTINUITY INFORMATION

This application is a::
> Application One::
Filing Date::

This application is a::
> Application Two::
Filing Date::

which is a::
>> Application Three::
Filing Date::

which is a::
>>> Application Four::
Filing Date::

PRIOR FOREIGN APPLICATIONS

Foreign Application One::
Filing Date::
Country::
Priority Claimed::